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Lignans from the plant species *Achillea lingulata**

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Abstract: Five lignans with a 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane skeleton, epieudesmin, kobusin, pinoresinol, fargesin and sesartemin, were isolated from the aerial parts and roots of *Achillea lingulata*. Their structures were identified by comparison of their ^{1}H-NMR and MS data to those in the literature. Fargesin and pinoresinol have not been isolated previously from any species of the genus *Achillea*.

Keywords: Asteraceae, *Achillea lingulata*, lignans, epieudesmin, kobusin, pinoresinol, fargesin, sesartemin.

INTRODUCTION

Genus *Achillea* L. (family Asteraceae) comprises about 100 species.[1] The aerial parts of different species of the genus are widely used in folk medicine due to numerous medicinal properties, such as anti-inflammatory, antispasmodic, antihemorrhoidal, stomachic, antiseptic and emmenagogue.[2–4]

Despite the widespread occurrence of lignans in the plant kingdom, they have so far been obtained from only three species of the genus *Achillea: A. gypsicola*,[5] *A. ptarmica*[6] and *A. holesericea*.[7]

Lignans are generally believed to be formed by the oxidative coupling of two coniferyl alcohol units, followed by further modification processes. The biosynthetic reactions are assumed to be enzyme-catalyzed since the majority of natural lignans are in an optically active form.[8]

Lignans are known to possess a variety of biological activities: antitumor, antimitotic, antiviral, as well as toxicity to fungi, insects and vertebrates, which make them interesting for further explorations.[9]

In the course of our investigations of the chemical composition of wild-growing species of the genus *Achillea* from Serbia and Montenegro, the aerial parts and roots of *A.*

* Dedicated to Professor Miroslav Gašić on the occasion of his 70th birthday.

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lingulata Waldst. et Kit have been examined. This subcarpatian floral element can be found throughout the highland regions of the Balkan peninsula (with the exception of Greece) and Rumania.[1] Secondary metabolites of *A. lingulata*, with exception of essential oils,[10] have not been studied before.

EXPERIMENTAL

General

^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on a Varian Gemini 2000 spectrometer in $CDCl_3$. Mass spectra were obtained on a Finnigan Mat 8230 (EI, 70 eV) and DCI (150 eV, isobutane). IR spectra were measured in the form of transparent films on a Perkin Elmer FT-IR Spectrometer 1725 X. Silica gel (0.063–0.200 mm) was used for column chromatography (CC). Silica gel G and silica gel F-254 were used for analytical (0.25 mm) and preparative (0.75mm) thin layer chromatography (TLC).

Plant material

The *Achillea lingulata* was collected during the flowering season in July 1998, at Zekova Glava, mountain Bjelasica, Montenegro. A voucher specimen (BEOU 15667) is deposited at the herbarium of the Department of Botany, Faculty of Biology, University of Belgrade.

Extraction and isolation

The aerial parts and roots were examined separately. The ground air-dried aerial parts (400 g) were extracted with petroleum ether-ether-MeOH (1 : 1 : 1; 3 1; 24 h; room temp.). The solvent was evaporated under reduced pressure and the obtained extract (19 g) was treated with MeOH (300 ml) in order to remove long-chain hydrocarbons. The MeOH soluble part, obtained by filtration, was evaporated *in vacuo* to yield a residue (16.9 g) in the form of a viscous brown oil. This was fractionated by silica gel dry-column flash chromatography; the elution was started with petroleum ether and then the polarity was gradually increased by addition of ether (to 100 %) and MeOH (to 30 %).

The fractions containing lignans (eluted with 80–100 % ether) were combined and chromatographed on silica gel using toluene-ether-MeOH (7.5 : 2.0 : 0.5) as the eluent. Fractions (220 × 10 ml) were collected and analysed by TLC and were subsequently combined into 12 fractions (F_1-F_{12}).

F_4 was purified by preparative TLC (CH_2Cl_2-MeOH, 99.6 : 0.4) to afford kobusin (**4**, 0.8 mg). Epieudesmin (**1**, 23 mg) was obtained from F_6 by crystallization from ether–MeOH (1 : 1). F_9 was rechromatographed by CC on silica gel followed by preparative TLC (CH_2Cl_2-MeOH, 97 : 3) to yield pinoresinol (**3**, 0.35 mg).

The powdered roots (54 g) were extracted with petroleum ether–ether–methanol (1 : 2 : 2). Evaporation of the solvent *in vacuo*, yielded a crude extract (1.3 g) which was divided into five fractions by dry-column flash chromatography on silica gel, using the following solvents: petroleum ether, CH_2Cl_2, ether, EtOAc, MeOH (in portions of 200 ml). The combined fractions eluted with ether and EtOAc afforded a residue (300 mg), which was further divided into 200 fractions on silica gel CC (CH_2Cl_2–MeOH, 99.3 : 0.7). Fraction 170, containing a mixture of lignans, was rechromatographed by silica gel CC (CCl_4–ether–MeOH, 7.0 : 2.8 : 0.2) to yield 50 fractions (F_1-F_{50}).

Fargesin (**2**, 1.5 mg) was obtained from F_{21}, while **1** (4 mg) and sesartemin (**5**, 3.4 mg) were isolated from F_{41} by prep. TLC (CCl_4–ether–MeOH, 7.0 : 2.8 : 0.2). F_{29} contained an inseparable mixture of **4** and **5**.

All lignans exhibited M^+ ions in EIMS and $(M+H)^+$ ions in DCIMS corresponding to their molecular formulas. The IR and ^{1}H-NMR data of **1** – **5** and the ^{13}C-NMR data of **1** were identical to those published.

RESULTS AND DISCUSSION

As described in the Experimental section, the combination of various chromatographic techniques (dry-column flash chromatography, silica gel CC and prep. TLC) applied to the extracts of the aerial parts of *Achillea lingulata* afforded

three bisepoxylignans, epiudesmin[5,7,11,12] (**1**), pinoresinol[13] (**3**) and kobusin[7,14] (**4**). A similar procedure applied to the extract of the roots revealed four lignans, **1**, **4**, fargesin[11,12] (**2**) and sesartemin[7,12] (**5**). All the isolated compounds were identified by comparison of their spectra to those published in the literature.

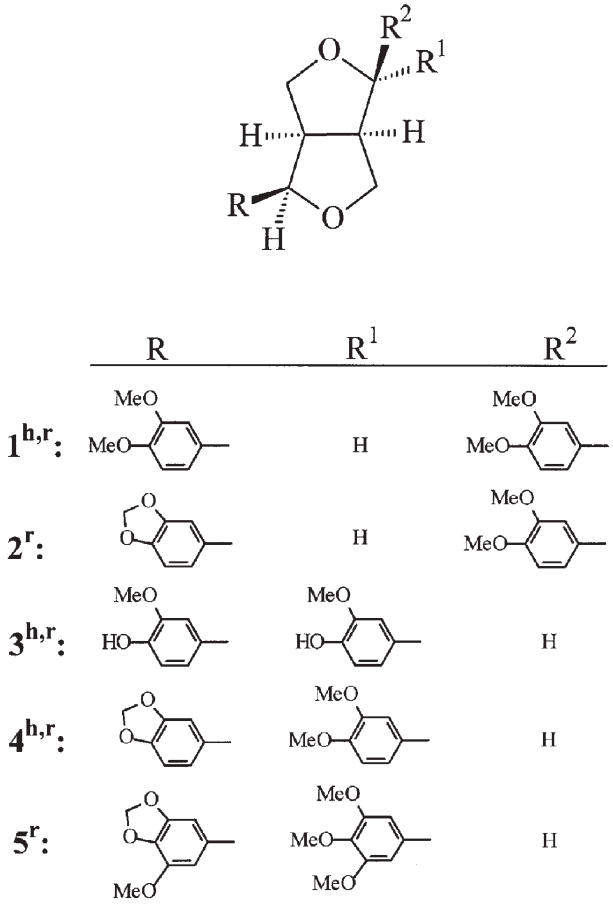

Fig. 1. Lignans from the aerial parts (h) and roots (r) of *Achillea lingulata*.

Whereas **2** and **3** have never been isolated previously from species of this genus, compounds **1**, **4** and **5** were found in the aerial parts of *Achillea holosericea* collected on the mountain Parnitha in Attiki (Greece).[7] Lignan **1** was also detected previously in the aerial parts of *Achillea gypsicola* from central Turkey.[5]

The ability of bisepoxylignans to influence enzyme activity has been studied before. They are effective inhibitors of cAMP phosphodiesterase, especially lignans containing a guaiacyl group in their structure (*e.g.*, **3** which was very active).[9] Due to their inhibitor efficiency on polysubstrate mono-oxygenases, some of them, *e.g.*, the diastereomer of **5** (diasesartemin) showed activity towards insects such as the European corn borer, *Ostrania nubilalis*.[15] Kobusin (**4**) inhibited the growth of silkworm larvae (*Bombyx mori*) while

some others showed efficiency in enhancing the toxicity of a wide variety of insecticides.[9] In addition, **2** exhibited platelet activating factor antagonist activity which is important because PAF was identified as a potent phospholipid mediator which may be involved in various inflammatory, respiratory and cardiovascular disorders.[16]

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ИЗВОД

ЛИГНАНИ БИЉНЕ ВРСТЕ *Achillea lingulata*

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Из надземног дела и корена биљне врсте *Achillea lingulata* изоловано је пет лигнана 2,6-диарил-3.7-диоксабицикло[3.3.0]октанског типа. То су епиеудесмин, кобусин, пинорезинол, фаргезин и сезартемин. Изоловани лигнани су идентификовани на основу идентичности њихових NMR и масених спектара са спектрима из литературе. Фаргезин и пинорезинол нису били до сада изоловани из биљних врста рода *Achillea*.

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